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MICROSCOPY.[1]

Fixation of the Methylenblue Stain.—In the last number of the *Zeitschrift für Wissenschaftliche Mikroskopie* (Vol. VIII., 1, 1891, p. 15), Prof. Dogiel offers some new points on the use of methylenblue in staining nerves.[2]

Nerves exposed to the direct action of methylenblue often stain so intensely that they appear dark blue, almost black. Such preparations should lie in the fixing medium (saturated aqueous solution of picrate of ammonium) twenty-four hours or more, otherwise the color fades quickly after transfer to glycerine. Long exposure to sunlight causes the stain to fade.

The fixing medium often macerates, loosening the epidermis and rendering difficult the investigation of intra-epithelial nerves. The macerating effects may be checked by adding osmic acid (1-2 cc. of a 1 per cent. solution to 100 cc. of the fixing fluid). This mixture preserves the tissue, and at the same time blackens the medullary sheaths of the nerve-fibers. As the axis cylinders stain dark violet, it becomes easy to distinguish medullated from non-medullated fibers. The preparations are mounted in dilute glycerine.

If the tissues are to be prepared for sectioning, a stronger per cent. of osmic is added (1-2 cc. of a 1 per cent. solution to 25–30 cc. of picrate of ammonium). The preparation lies in this fluid twenty-four hours, and is then cut in liver or pith, or with the freezing microtome.

Biedermann[3] recommends as a fixative in the case of invertebrates the following mixture:

Saturated aqueous solution of picrate of ammonium	1 vol.
Glycerine	1 vol.
Solution of salt, ½ per cent. (sea-water in case of marine forms)	2 vols.

The nervous system is dissected out for exposure to the staining fluid. That the exposure may be as direct as possible, the nerve-cord (Hirudo, Lumbricus, etc.) should be freed from its sheath. A very dilute solution of methylenblue is used, and allowed to act from two to three hours. If the preparation, after the exposure, be placed on a slide wet with the staining fluid, and left for one-half to one hour in a moist chamber, the color effects will be intensified. This

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[1] Edited by C. O. Whitman, Clark University. Worcester, Mass.

[2] Cf. this journal, Dec. 1890, p. 1219.

[3] *Jenaische Zeitschrift für Naturwissenschaft*, XXV., 3 and 4, 1891, p. 433.

"airing" is not required with marine animals, as with them the color differentiation is completed in the staining fluid.

In the case of marine animals the methylenblue is dissolved in seawater. It dissolves less readily than in fresh water, and owing to this weak solubility it is liable to form a fine granular or crystalline precipitate on the surface of the preparation. As a large part of the dissolved staining substance is lost by filtering, it is best to prepare it fresh each time, and to allow it to settle, so that the clear fluid can be turned off for use. In the case of Nereis the nerve-cord is not obscured by a thick opaque sheath, and hence it is only necessary to open the dorsal wall lengthwise and spread it out flat in order to apply the stain.

Vasale's Modification of Weigert's Method.[4]—The pieces of the nervous system to be prepared are hardened in Müller's fluid or in bichromate of potash, and then, either with or without washing, left in alcohol until they are wanted for sectioning. For staining, the three following fluids are required :

1. Hæmatoxylin 1 g., dissolved in 100 g. water by heating.
2. Neutral acetate of copper, saturated, filtered solution.
3. Borax 2 g., ferricyanide of potash 2½ g., dissolved in 300 g. water.

The sections taken from alcohol are placed in solution 1 for three to five minutes; then for the same time in solution 2, in which they become black. They are next washed quickly in water and put into solution 3, which is stirred, and in which the ganglion-cells, neuroglia, and the degenerated parts are quickly discolored, while the medullated fibers remained stained dark violet. Finally the sections are washed in water and quickly placed in absolute alcohol. They are cleared with *carbol-xylol* (three parts xylol to one of liquid carbolic acid), and mounted in xylol balsam. This clearing mixture has the advantage that it does not shrink sections inclosed in celloidin. A contrast-stain may be obtained if the sections, after being washed, are treated with alum carmine or picrocarmine, or according to Pal's method.

Upson's Gold-Staining Method for Axis-Cylinders and Nerve-Cells.[5]—Pieces of the central nervous system are hardened in potassium bichromate, beginning with one per cent. and increasing to 2½ per cent. They are left in this fluid in the dark from four to six months, then washed quickly and placed in alcohol of 50 per cent. two to three days, which should be renewed a few times. They are then transferred to 95 per cent. alcohol, and left until they show plainly a greenish color (two to four weeks or more).

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[4] Rivista sperimentale di Freniatria e di Medicina legale, Vol. XV., 1889., p. 102–5. *Zeitschr. f. Wiss. Mikr.*, VII., 4, 1891, p. 518.

[5] A. Mercier. "Die Upson'schen Methoden für Achsencylinder und Zellen (Gold) Färbung. *Zeitschr. f. Wiss. Mikr.*, VII., 4, p. 474, 1891.

The preparations are then ready for sectioning, and may be cut without imbedding, or after imbedding in celloidin. Two methods of staining are used:

1. The section is placed in a one per cent. solution of gold chloride, to which two per cent. hydrochloric acid has been added (about two hours), then washed in distilled water, and transferred to a ten per cent. solution of potash for half a minute.

The section is then washed in distilled water, and there left until the following reducing fluid (to be made fresh each time) is prepared:

Acid. sulfurosum	5 cc.
Tinct. iodi., 3 per cent.	10–15 drops.

Mix, and add

Liq. ferrichlorid.	1 drop.

This fluid must be made very exactly and quickly, and the moment it is ready the section is placed in it and left until it assumes a rose color. If left only a few seconds too long it becomes dark red, and is worthless.

As soon as the proper color appears the section is transferred to distilled water, and then to absolute alcohol on the slide. After ten to fifteen minutes it may be cleared in clove oil and mounted in balsam. The slide is to be kept in the dark.

2. The section is placed for two hours in the following solution:

Gold chloride, 1 per cent.	5 cc.
Ammonium vanadicum, saturated solution	10 drops.
Acid hydrochlor.	3 drops.

Wash, and transfer to the following (freshly prepared) mixture for ½ to 1 minute:

Caustic potash, 10 per cent.	5 cc.
Ammonium vanadicum	a trace.
Permanganate of potash, 10 per cent.	10 drops.

Wash, and expose to the following reducing fluid, freshly prepared:

Tin solution	15 drops.
Aq. dest.	3 cc.
Iron solution	3–5 drops.
Acid. sulfurosum	3 cc.

The moment the acid sulfurosum is added a thick precipitate arises, and at this moment the reducing fluid is strongest.

The method of handling the section is the same as in method 1.

The tin solution is made by adding so much chloride of tin to three per cent. tincture of iodine until the color is white or yellowish. The iron solution is a saturated solution of ferrum phosphoricum in distilled water.

The method is somewhat cumbersome, but the results are said to be extraordinary.

Method of Preparing Rotifers.[6]—The first difficulty which one experiences in studying the rotifers is their constant motion. This difficulty is overcome, according to Masius, by the use of a mixture of methyl alcohol, water, and cocaine in weak solution.

After being anæsthetized by this fluid, the rotifers may be fixed without contraction in the ordinary preservative fluids,—Flemming's fluid, for example.

For the study of the head, an anæsthetized specimen is placed upon a slide, and the head cut off in a transverse plane as near as possible to the anterior end. The section thus obtained can be examined easily from any side in water or weak alcohol.

PROCEEDINGS OF SCIENTIFIC SOCIETIES.

Natural Science Association of Staten Island.—April 11th.—Mr. Arthur Hollick stated that a nest of the barred owl (*Syrnium nebulosum*) was found on Staten Island, March 27th, by Mr. Chas. Rufus Harte, a student of Columbia College, This is an addition to the list of birds known to breed here, and the following memorandum from Mr. Harte was read:

"In the woods which are the continuation of the swamp (near Bull's Head) I came upon a sweet gum, having an 'owlish' looking cavity. As I turned aside to investigate, a barred owl flew out and away into the depths of the woods, appearing again once or twice, but always at a very respectable distance. The tree was about two feet in diameter, with no limbs below the opening, which was some thirty feet up, and very irregular in form. The cavity into which it opened was about eight inches in diameter, and was filled to within six inches of the mouth with dead leaves and feathers. On this bed lay the three

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[6] *Arch. de Biol.*, X., 4, 1890, p. 652.